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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

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Applicant's or agent's file reference P864PC00	FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)	
International application No. PCT/DK 03/00326	International filing date *(day/month/year)* 15.05.2003	Priority date *(day/month/year)* 17.05.2002
International Patent Classification (IPC) or both national classification and IPC C07K14/15		
Applicant PIPELINE BIOTECH A/S et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 4 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 5 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
16.12.2003	31.08.2004
Name and mailing address of the international preliminary examining authority: European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized Officer Niemann, F Telephone No. +31 70 340-1088

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/DK 03/00326

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-48 as originally filed

Sequence listings part of the description, Pages

1-3 as originally filed

Claims, Numbers

1-40 received on 16.08.2004 with letter of 16.08.2004

Drawings, Sheets

1/6-6/6 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☒ contained in the international application in written form.

   ☒ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☐ furnished subsequently to this Authority in computer readable form.

   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application,

   ☒ claims Nos. 1-3

   because:

   ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (specify):

   ☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☒ no international search report has been established for the said claims Nos. 1-3

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the Standard.

   ☐ the computer readable form has not been furnished or does not comply with the Standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	4-40
	No:	Claims	
Inventive step (IS)	Yes:	Claims	4-40
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	4-40
	No:	Claims	

2. Citations and explanations

   see separate sheet

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The International Search Report has not been established for claims 1-3.
Consequently, said claims need not be the subject of international preliminary examination (Rule 66.1(e) PCT). The examination was carried out as if claims 1-3 were not present.

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Because of the large number of independent claims, the claims as a whole lack conciseness and clarity and as such do not meet the requirements of Article 6 PCT.

Subject-matter of claims 21-22 is new in the sense of Article 33(2) PCT, the scope of claims 21-22 does not encompass the well known wild type virus MLV strain SL3-2.

The present application does meet the criteria of Article 33(1) PCT, because the subject-matter of claims 4-40 does involve an inventive step in the sense of Article 33(3) PCT.
One of the problems to be solved by the present application may be seen as providing a purified polypeptide, which is capable of mediating infection of a cell, by use of the polytropic/xenotropic receptor encoded by the Rmcl locus from a NIH Swiss inbred NFS/N mouse for entry, and unable of mediating infection of a cell by use of a human polytropic/xenotropic receptor encoded by the human RMC1 locus. The solution proposed is the subject matter of claim 4.
Another problem to be solved by the present application may be seen as providing mutated envelope proteins from the MLV strain SL3-2 capable of mediating infection of a human cell. The solution proposed is the subject matter of claim 5.
Both solutions are considered as involving an inventive step as nothing in the prior art is suggesting the claimed solutions, nothing is mentioning the effect on the host tropism of mutations in VR3 region of the envelope protein of the strain SL3-2.
Subject matter of claim 18 is considered as involving an inventive step as isolated nucleic acid sequence of SL3-2 as shown in SEQ ID NO : 1 encodes the envelope protein from the MLV strain SL3-2 which is the solution of the first above mentioned problem.

Claims

1. A purified retroviral envelope polypeptide, capable of mediating infection of a cell by use of the polytropic/xenotropic receptor encoded by the Rmc1 locus of the NIH *Swiss* inbred NFS/N mouse for entry, and unable of mediating infection of a cell by use of a human polytropic/xenotropic receptor encoded by the human RMC1 locus.

2. A purified retroviral envelope polypeptide, capable of mediating infection of a cell derived from *Mus musculus* by use of the polytropic/xenotropic receptor encoded by the Rmc1 locus isolated from a NIH *Swiss* inbred NFS/N mouse for entry, and unable of mediating infection of a human cell solely expressing a human polytropic/xenotropic receptor encoded by the human RMC1 locus.

3. A purified murine retroviral envelope polypeptide, capable of mediating infection of a cell derived from *Mus musculus* using the polytropic/xenotropic receptor encoded by the Rmc1 locus from a NIH *Swiss* inbred NFS/N mouse for entry, and unable of mediating infection of a human cell comprising a human polytropic/xenotropic receptor encoded by the human RMC1 locus.

4. A purified retroviral envelope polypeptide comprising an amino acid sequence which is at least 94% identical to the amino acid sequence shown in SEQ ID NO: 2, or a fragment of said amino acid sequence that is at least 94% identical to the sequence shown in SEQ ID NO: 2.

5. A purified retroviral envelope polypeptide according to claim 4, wherein said polypeptide includes at least one substitution in the VR3 region.

6. A purified retroviral envelope polypeptide according to claim 5, wherein said mutation is at position 212 in SEQ ID NO: 2.

7. A purified retroviral envelope polypeptide according to claim 5 or 6, wherein said at least one substitution alters the host tropism of a virus or an infectious particle comprising said polypeptide.

8. A purified retroviral envelope polypeptide according to any of claims 5-7, wherein said purified polypeptide is a murine retroviral envelope polypeptide capable of mediating infection of a human cell.

9. A purified retroviral envelope polypeptide according to any of claims 5-8, wherein said mutation at position 212 in SEQ ID NO: 2 results in a methionine.

10. A purified retroviral envelope polypeptide according to any of claims 5-9 capable of mediating a higher infectivity in human cells than MCF-247, MCF-13 and X-MLV (NZB) viruses.

11. A purified retroviral envelope polypeptide according to any of claims 1-10, further comprising an inserted non-viral sequence capable of redirecting the target cell specificity, by the resultant chimeric envelope.

12. A purified retroviral envelope polypeptide according to claim 11, wherein the chimeric envelope further contains secondary mutations, enabling activation of the fusiogenic properties of said chimeric envelope, by binding to the receptor target.

13. A purified retroviral envelope polypeptide according to any of claims 11 and 12, wherein said inserted sequence is a single chain antibody.

14. A purified retroviral envelope polypeptide according to any of claims 1-13, further comprising a chemical modification of said envelope.

15. A purified retroviral envelope polypeptide according to claim 14, wherein said chemical modification enhances and/or alters the host tropism.

16. A recombinant mammalian cell displaying an envelope polypeptide according to any of claims 1-15.

17. An isolated nucleic acid sequence encoding any of the envelope polypeptides according to any of claims 4-10.

18. An isolated nucleic acid sequence as shown in SEQ ID NO: 1

19. A recombinant mammalian expression vector comprising a purified envelope polypeptide according to claims 1-4 and/or 11-15.

20. A recombinant mammalian expression vector comprising a purified envelope polypeptide according to claims 5-10 and/or 11-15.

21. A replication competent retrovirus comprising a purified envelope polypeptide according to any of claims 1-4 and/or 11-15.

22. A replication competent retrovirus, comprising a purified envelope polypeptide according to any of claims 5-10 and/or 11-15.

23. A vector according to 21 and 22 further comprising a heterologous translation cassette.

24. A vector according to claim 23, wherein said heterologous translation cassette consists of an IRES-gene element.

25. A retroviral expression vector comprising a purified envelope polypeptide according to claims 1-4 and/or 11-14.

26. A retroviral expression vector comprising a purified envelope polypeptide according to claims 5-10 and/or 11-15.

27. A vector according to claim 19 or 20 or 25-26, further comprising at least one heterologous gene to be expressed.

28. A vector according to claim 27, wherein expression of the envelope is directed by a IRES-element.

29. A packaging cell construct comprising a recombinant mammalian expression vector comprising a nucleic acid coding for a purified envelope polypeptide according to any of claims 1-15, and a non-viral or viral promoter and poly-adenylation signals.

30. Use of a vector according to any of claims 19-20 and/or 29 for the generation of a packaging cell.

31. Use of a vector according to any of claims 25-28, for expression in a cell constitutively expressing the gag/pol genes of simple retroviruses.

32. Use of a packaging cell according to any of claims 29-31 for the preparation of a composition for the modification of a cell.

33. Use of a vector according to claims 23 and/or 24, for the preparation of a composition for the modification of a cell.

34. Use of a virus or vector according to any of claims 19-28 for gene discovery by infection of a new-born rodent.

35. Use of a virus or vector according to claim 34, wherein said rodent constitutively express the gag/pol genes of simple retroviruses.

36. Use of a virus or vector according to claim 34, wherein said rodent express the gag/pol genes of simple retroviruses in a tissue specific manner.

37. Use of a virus or vector according to claim 34, wherein said rodent expression of the gag/pol genes of simple retroviruses is developmentally regulated.

38. Use of a virus or vector according to claim 34, wherein said rodent expresses the gag/pol genes of gamma retroviruses tissue specifically and in a developmentally regulated manner.

39. A method for gene discovery comprising

a) using a virus or vector according to any of claims 19-28

b) infecting a new-born rodent with said virus or vector

c) inducing a tumour by means of said virus or vector

d) isolating said tumour in said rodent

e) identifying a gene involved in the oncogenesis by cloning the integration site of said virus or vector in said tumour.

40. A method according to claim 39 for gene discovery of a cancer related gene.

41. Use of any of the envelope polypeptides according to claims 1-4 and/or 10-14 or vectors comprising said polypeptides in a bio-safety level 1/PS I/SI laboratory animal facilities or equivalents thereof.

INTERNATIONAL SEARCH REPORT

PCT/DK 03/00326

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07K14/15 C12N15/867

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
CHEM ABS Data, SEQUENCE SEARCH, WPI Data, SCISEARCH, EMBASE, BIOSIS, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	PEDERSEN F S ET AL: "NOVEL LEUKEMOGENIC RETROVIRUSES ISOLATED FROM CELL LINE DERIVED FROM SPONTANEOUS AKR TUMOR" NATURE (LONDON), vol. 292, no. 5819, 1981, pages 167-170, XP002220719 ISSN: 0028-0836 cited in the application the whole document --- -/--	

[X] Further documents are listed in the continuation of box C. [ ] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 August 2003	26/08/2003

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Niemann, F

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	DAI H Y ET AL: "Multiple sequence elements in the U3 region of the leukemogenic murine retrovirus SL3-2 contribute to cell-dependent gene expression." VIROLOGY. UNITED STATES APR 1990, vol. 175, no. 2, April 1990 (1990-04), pages 581-585, XP008010442 ISSN: 0042-6822 cited in the application the whole document	
A	REIN A ET AL: "DIFFERENT RECOMBINANT MURINE LEUKEMIA VIRUSES USE DIFFERENT CELL SURFACE RECEPTORS" VIROLOGY, RAVEN PRESS, NEW YORK, NY, US, vol. 136, 1984, pages 144-152, XP000569912 ISSN: 0042-6822 cited in the application the whole document	

INTERNATIONAL SEARCH REPORT

PCT/DK 03/00326

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [ ] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. [X] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Present claims 1-3,11-16,19-41 relate to a purified retroviral envelope polypeptide defined by reference to a desirable characteristic or property, namely being capable of mediating infection of a cell by use of the polytropic/xenotropic receptor encoded by the Rmc1 locus of the NIH Swiss inbred NFS/ mouse for entry, and unable of mediating infection of a cell by use of a human polytropic/xenotropic receptor encoded by the human RMC1 locus

The claims cover all purified retroviral envelope polypeptides having this characteristic or property, whereas the application provides support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for only a very limited number of such purified retroviral envelope polypeptide. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity. An attempt is made to define the purified retroviral envelope polypeptide by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible. Consequently, the search has been carried out for those parts of the claims which appear to be clear, supported and disclosed, namely those parts relating to the purified retroviral envelope polypeptide comprising the amino acid sequence shown in SEQ ID NO: 2 (see claim 4).

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.